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Synthesis of the Octa- and Nonachlorobiphenyl Isomers and Congeners and their Quantitation in Commercial Polychlorinated Biphenyls and Identification in Human Breast Milk

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Abstract

The synthesis of all possible isomeric nona- and octachlorobiphenyls has been accomplished by the Cadogan coupling of commercially available or synthetic chlorinated anilines in the presence of excess chlorinated benzenes and isoamyl nitrite. 2,3,4,6-Tetrachloroaniline was prepared by the chlorination of 2,4,5-trichloroaniline. The synthetic polychlorinated biphenyls (PCBs) were characterized by their proton magnetic resonance and mass spectra and their purities determined by gas chromatographic analyses. The PCB standards were used to unambiguously identify the deca-, nona-, and octachlorobiphenyls present in human breast milk and in the commercial PCB preparations Aroclors 1268, 1262, 1260, 1254, 1248, 1242, 1016, 1232 and 1221 utilizing high resolution glass capillary gas chromatogrpahy.

Introduction

Polychlorinated biphenyls (PCBs) are industrial chemicals which are prepared by the chlorination of biphenyl and the commercial products are classified according to their chlorine content. The chlorination process is not highly site specific and therefore most of the commercial products are complex mixtures of chlorinated biphenyl isomers and congeners (1-5). The diverse industrial applications of PCBs are directly related to their physical and chemical properties which include: resistance to acids and bases, resistance to oxidation and reduction, solubility in organic solvents, excellent dielectric insulation properties, thermal stability and non-flammability. These properties, coupled with their widespread use, has resulted in the detection of PCB residues in almost every component of the global ecosystem (6). Moreover the lipophilic nature of PCBs contributes to their bioconcentration in the food chain (6).

The quantitative and qualitative gas chromatographic analysis of the commercial PCBs is complicated by the complex composition of these products and the fact that the identities of many of the individual components are unknown. Due to the different physico-chemical and biological properties of the individual PCB congeners, the composition of atmospheric and the aquatic PCBs, and residues in biological tissues are different from that of the parent commercial products (7-9). Therefore, information concerning the precise composition of these mixtures is required for an understanding of the toxicity of residual PCBs. This study reports the chemical synthesis and characterization of all the possible nona- and octachlorobiphenyls and quantitates their levels and the level of decachlorobiphenyl in several commercial PCB preparations and a human breast milk sample.

Materials and Methods

Chemical Sources and Synthesis

Pentachloraniline, 2,3,4,5- and 2,3,5,6-tetrachloroaniline, 2,3,4-, 2,4,5- and 3,4,5-trichloroaniline, pentachlorobenzene, 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene, 1,3,5- and 1,2,4-trichlorobenzene were purchased from the Aldrich

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Chemical Company* (Milwaukee, Wisconsin). Isoamyl nitrite was obtained from Eastman Organic Chemicals (Rochester, New York). The commercial Aroclors were either a gift of Monsanto Chemical Company (St. Louis, Missouri) or purchased from RFR Corporation (Hope, Rhode Island) or Supleco (Bellefonte, Pennsylvania). All the PCB-isomers and congeners were synthesized via the Cadogan coupling (10) of a chlorinated aniline (10-15 mmol) in excess of an appropriate chlorinated benzene (100-200 mmol) as indicated in Table I. Isoamyl nitrite (20-25 mmol) was added to the stirred solution of the reactants at 120-130°C over a period of 60 minutes and the reaction was continued for an additional 18 hours. After removal of the excess unreacted chlorinated benzene, the crude product was adsorbed onto neutral alumina or silicic acid, added to a column of Florisil with a top layer of neutral alumina and the PCB product was eluted with petroleum spirit (b.p., 30-60°C, 300-400 mL). The eluate was concentrated and the residue further purified by preparative thin-layer chromatography (TLC) on silica gel HF_{254} plates (0.9 mm thickness, Merck). All of the reactions listed in Table I, with the exception of the condensation of pentachloroaniline and 1,2,4-trichlorobenzene, gave one major reaction product. The TLC purified material was crystallized from methanol and their purities determined by packed-column gas chromatography (GC) using a Hewlett-Packard Model 5710 chromatograph, equipped with a ^{63}Ni electron capture (EC) detector and an 0.6 cm x 1.2 m glass column packed with 3% OV 101 on Ultrabonded Carbowax 20 *M* 80/100 mesh.

The reaction of pentachloroaniline with 1,2,4-trichlorobenzene gave three products and after repeated preparative TLC, two major bands were separated; the GLC and proton magnetic resonance (PMR) spectra of the top band confirmed that this compound was the 2,2′,3,3′,4,5,6,6′-octachlorobiphenyl congener (see Table I). The second band was a mixture of the 2,2′,3,3′,4,5,5′,6- and 2,2′,3,4,4′,5,5′6-octachlorobiphenyl isomers. However, since the latter isomer was prepared as a single product by the condensation of 2,4,5-trichloroaniline and pentachlorobenzene, the GC retention time and PMR spectra of 2,2′,3,3′,4,5,5′,6-octachlorobiphenyl were readily deduced from the mixture.

2,3,4,6-Tetrachloroaniline was readily prepared by the reaction of 2,4,5-trichloroaniline with a mole equivalent of chlorine in refluxing carbon tetrachloride solution. The reaction has to be continually monitored by GC to avoid overchlorination. 2,3,4,6-Tetrachloroaniline gave PMR (acetone d_6) δ 7.46 (s, H-5 aromatic) ppm and M^+ 229. The PMR spectra were recorded on a Bruker multinuclear 60 MHz PMR spectrometer in acetone d_6 and are summarized in Table I. The molecular weights of all the PCBs were confirmed by their mass spectra, which were obtained on a VG micromass 7070 high resolution mass spectrometer.

High resolution glass capillary gas chromatograms were obtained using a Varian 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector (EC). The PCBs were separated on a 0.25 mm i.d. x 56 m glass capillary column coated with C-87 (11) to a thickness of 0.2 - 0.3 μm (QuadRex Corporation, New Haven, Connecticut). The oven temperature was programmed at a rate of 1.3°C/min from 140 to 230°C. The injector was held at 300°C and the EC detector was held at 320°C. The electrometer was at 8 x 1 attenuation. Sample volume injected was 2.5 μL utilizing an automatic sampler with no sample splitting in the injector. The hydrogen carrier gas was held at a constant pressure of 1.76 kg/cm² to give the optimized linear velocity ($\bar{u}$) at 100°C of 45 cm/sec. The advantages of capillary gas chromatography over packed-column gas chromatography are discussed elsewhere (3,12).

Results and Discussion

Synthesis and Physico-Chemical Properties of PCB Congeners

The Cadogan coupling reaction was used to prepare all the nona and octachlorobiphenyl isomers which were readily

*The mention of brand or trade names does not constitute endorsement by the U.S. Environmental Protection Agency.

Table I. Synthetic Precursors and Spectral Properties of the Octa- and Nonachlorobiphenyls

PCB Congener Substitution Pattern	Synthetic Precursors	Proton Magnetic Resonance Spectral Shifts (ppm)
Octachlorobiphenyls*		
194 2,2′,3,3′,4,4′,5,5′	2,3,4,5-tetrachloroaniline, 1,2,3,4-tetrachlorobenzene	7.75 (H-6, H-6′, s)
195 2,2′,3,3′,4,4′,5,6	2,3,4-trichloroaniline, pentachlorobenzene	7.46 (H-6′, d, J=8.3 Hz), 7.82 (H-5′, d, J=8.3 Hz)
196 2,2′,3,3′,4,4′,5,6′	2,3,4,5-tetrachloroaniline, 1,2,3,5-tetrachlorobenzene	7.77 (H-6, s), 7.94 (H-5′, s)
197 2,2′,3,3′,4,4′,6,6′	2,3,4,6-tetrachloroaniline, 1,2,3,5-tetrachlorobenzene	7.99 (H-5, H-5′, s)
198 2,2′,3,3′,4,5,5′,6	pentachloroaniline, 1,2,4-trichlorobenzene	7.56 (H-6′, d, J=2.2 Hz), 7.87 (H-4′, d, J=2.2 Hz)
199 2,2′,3,3′,4,5,6,6′	pentachloroaniline, 1,2,4-trichlorobenzene	7.67 (H-5′, d, J=8.4 Hz), 7.87 (H-4′, d, J=8.4 Hz)
200 2,2′,3,3′,4,5′,6,6′	2,3,5,6-tetrachloroaniline, 1,2,3,5-tetrachlorobenzene	8.01 (H-5, s), 8.11 (H-4′, s)
201 2,2′,3,3′,4,5,5′,6′	2,3,5,6-tetrachloroaniline, 1,2,3,4-tetrachlorobenzene	7.79 (H-6, s), 8.06 (H-4′, s)
202 2,2′,3,3′,5,5′,6,6′	2,3,5,6-tetrachloroaniline, 1,2,4,5-tetrachlorobenzene	8.12 (H-4, H-4′, s)
203 2,2′,3,4,4′,5,5′,6	2,4,5-trichloroaniline, pentachlorobenzene	7.74 (H-6′, s), 7.94 (H-3′, s)
204 2,2′,3,4,4′,5,6,6′	pentachloroaniline, 1,3,5-trichlorobenzene	7.79 (H-3′, H-5′, s)
205 2,3,3′,4,4′5,5′,6	3,4,5-trichloroaniline, pentachlorobenzene	7.66 (H-2′, H-6′, s)
Nonachlorobiphenyls*		
206 2,2′,3,3′,4,4′,5,5′,6	2,3,4,5-tetrachloroaniline, pentachlorobenzene	7.79 (H-6′, s)
207 2,2′,3,3′,4,4′,5,6,6′	pentachloroaniline, 1,2,3,5-tetrachlorobenzene	8.02 (H-5′, s)
208 2,2′,3,3′,4,5,5′,6,6′	2,3,5,6-tetrachloroaniline, pentachlorobenzene	8.13 (H-4′, s)

*Molecular ion (M^+)

separated by TLC and identified by PMR. The condensation of pentachloroaniline with 1,2,4-trichlorobenzene gave a mixture of three octachloro isomers and TLC gave the 2,2′3,3′,4,5,6,6′-octachlorobiphenyl as a single product characterized by the *ortho* coupling of H-4 and H-5. The 2,2′,3,3′,4,5,5′,6- and 2,2′,3,4,4′,5,5′,6- isomers were not separable by TLC, however, the PMR spectra exhibited the expected two singlets at 7.74 and 7.94 ppm for H-3 and H-6 for the latter compound the two *meta*-coupled (J = 2.2 Hz) doublets for H-4′ and H-6′ for the former PCB, respectively. These compounds had to be prepared as a mixture because the corresponding aniline precursors, 2,3,5- and 2,3,6-trichloroaniline, are not commercially available nor readily synthesized. The only tetrachloroaniline which could not be purchased was the 2,3,4,6-tetrachloro isomer and this was readily synthesized by the monochlorination of 2,4,5-trichloroaniline. This intermediate was required for the synthesis of 2,2′,3,3′,4,4′,6,6′-octachlorobiphenyl.

The PMR spectra of the PCBs were readily interpreted and the chemical shift values exhibited the following trends:

(i) the *ortho* hydrogen atoms H-2(2′) and H-6(6′) protons appeared at highest field and were the most shielded aromatic protons;

(ii) the *para* protons, H-4 consistently appeared at lowest field and were the most deshielded;

(iii) the *meta* proton (H-3 and H-5) chemical shifts were invariably at values between the *ortho* and *para* aromatic protons.

High Resolution Glass Capillary Gas Chromatography

The effects of structure on the capillary gas chromatographic retention times were relatively consistent throughout the octachlorobiphenyl series, that is:

(i) the retention times increased with decreasing *ortho* chlorine substitution (C-2,2′,6.6′);

(ii) given the same *ortho* substitution pattern, increasing *para* (4 and 4′) chloro substitution (and decreasing *meta* [3,3′,5 and 5′] substitution) increases the relative capillary GC retention times;

(iii) the effects of the relative degrees of phenyl ring chorination are not readily assessed (*i.e.*, a 5:3 versus a 4:4 pattern); however, the most polar isomer, 2,3,3′,4,4′,5,5′,6-octachlorobiphenyl contains a 3,3′,4,4′5,5′- substitution pattern; this substitution of the lateral *meta* and *para* positions in biphenyl always gives the most polar isomers in a series, that is; 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl and 3,3′,4,4′,5,5′-hexachlorobiphenyl, are the most polar compounds within their respective isomeric groups (2);

(iv) the correlations noted above are also prevalent for nonachlorobiphenyl isomers;

(v) the relative electron capture detector response factors for the octa, nona and decachlorobiphenyls vary over a 2.5-fold range and there are no apparent structural features which correlate with relative capture response sensitivities.

Table II. Relative Retention Times and Response Factors of the Deca-, Nona- and Octachlorobiphenyls and Their Quantitation in Commercial PCBs.

Peak No.	Isomer	Relative[a] Retention Time	Detector[b] Response Factor (pb^{-1})	1268	1262	1260	1254	1248	1242	1016	1232	1221
Octachlorobiphenyls												
194	2,2′,3,3′,4,4′,5,5′	1.546	2.644	4.74	3.97	2.56	TR	TR	TR	ND	TR	ND
195	2,2′,3,3′,4,4′,5,6	1.395	2.227	0.28	1.28	0.90	TR	TR	TR	TR	TR	ND
196	2,2′,3,3′,4,4′,5,6′	1.334	1.249	1.91	2.47	1.58	ND	TR	TR	ND	ND	ND
197	2,2′3,3′,4,4′,6,6′	1.184	1.409	TR[c]	TR	TR	TR	TR	TR	TR	TR	TR
198	2,2′,3,3′,4,5,5′,6	1.312	1.377	ND[d]	ND	ND	ND	ND	ND	ND	ND	ND
199	2,2′,3,3′,4,5,6,6′	1.175	1.156	1.09	0.95	0.61	0.26	ND	ND	ND	ND	ND
200	2,2′,3,3′,4,5′,6,6′	1.148	1.761	9.09	5.96	3.64	ND	ND	ND	ND	ND	ND
201	2,2′,3,3′,4,5,5′,6′	1.306	1.043	18.76	6.82	3.55	TR	TR	TR	TR	TR	TR
202	2,2′,3,3′,5,5′,6,6′	1.111	1.007	5.95	1.25	0.58	ND	ND	ND	ND	ND	ND
203	2,2′,3,4,4′,5,5′,6	1,348	2.393	11.66	3.09	1.70	TR	TR	ND	ND	ND	ND
204	2,2′,3,4,4′,5,6,6′	1.179	1.954	ND	ND	ND	ND	ND	ND	ND	ND	ND
205	2,3,3′,4,4′,5,5′,6	1.596	2.531	0.37	0.28	0.20	ND	ND	ND	ND	ND	ND
Nonachlorobiphenyls												
206	2,2′,3,3′,4,4′,5,5′,6	1.684	2.474	22.06	0.28	0.50	ND	TR	ND	ND	ND	ND
207	2,2′,3,3′,4,4′,5,6,6′	1.417	1.799	3.14	0.29	0.13	ND	ND	ND	ND	ND	ND
208	2,2′,3,3′,4,5,5′,6,6′	1.370	2.128	8.22	0.27	0.10	ND	TR	ND	ND	ND	ND
Decachlorobiphenyl												
209	2,2′,3,3′,4,4′,5,5′,6,6′	1.773	2.513	7.67	ND	ND	ND	ND	ND	ND	ND	ND
Total Percent[e]				94.97	26.96	16.09	0.49	0.45	0.21	0.08	0.15	0.08

[a] 2,2′,4,4′,5,5 -hexachlorobiphenyl = 1.000
[b] 1 picogram 2,2′,4,4′,5,5′-hexachlorobiphenyl = 1.000
[c] TR = Trace (0.01% ≤ x < 0.10%)
[d] ND = Not detected (<0.01%)
[e] Sum of percentages of octa-, nona- and decachlorobiphenyls in each Aroclor

The higher chlorinated commercial Aroclors 1268, 1262, and 1260 contain variable amounts of all the nona- and octachlorobiphenyls with the exception of 2,2',3,3',4,4',6,6'- (traces), 2,2',3,3',4,5,5',6- (not detected) and 2,2',3,4,4',5,6-6'- (not detected) octachlorobiphenyls. The major components in all three preparations were the 2,2',3,3',4,5,5',6'-, 2,2',3,3',4,5',6,6'-, 2,2',3,4,4',5,5',6- and 2,2',3,3',4,4',5,5'-octachlorobiphenyl isomers. The major nonachlorobiphenyl was the 2,2',3,3',4,4',5,5',6- isomer present in Aroclor 1268 (7.67%). Aroclors 1254, 1248, 1242, 1016, 1232 and 1221 contained trace levels (<0.50%) of these higher chlorinated biphenhyls. Although the decachlorobiphenyl congener was detected by the signal integrator only in Aroclor 1268, visual inspection of the capillary chromatograms of Aroclors 1260 and 1262 evinces the presence of this congener in these commercial mixtures. Table II summarizes the relative retention times and response factors of these congeners in all the PCB Aroclor formulaitons.

Figures 1 and 2 are the high resolution glass capillary gas chroamtograms [$(GC)^2$] of Aroclor 1260 and a human milk sample extract, respectively, with the peaks identified according to the numbering scheme in reference 3. The identification of the PCB isomers is facilitated by the use of $(GC)^2$ with each compound being resolved from adjacent peaks. Peaks corresponding to additional compounds of interest in the milk sample are noted in Figure 2. Current research on the unambiguous synthesis of lower chlorinated PCB isomers and congeners is in progress and it is anticipated that these components will facilitate identification of PCB in the commercial mixtures and environmental samples.

Acknowledgements

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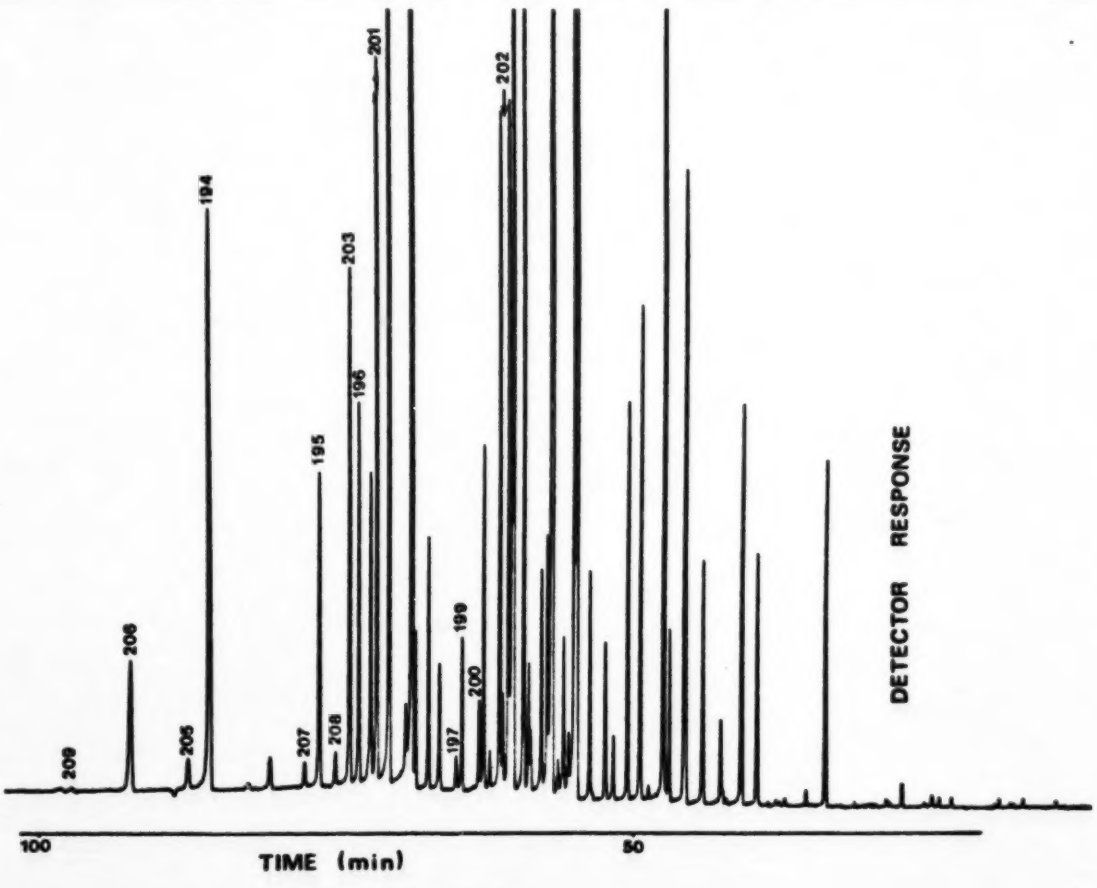

Figure 1. Glass capillary gas chromatogram of Aroclor 1260 (see text for instrumental operating conditions).

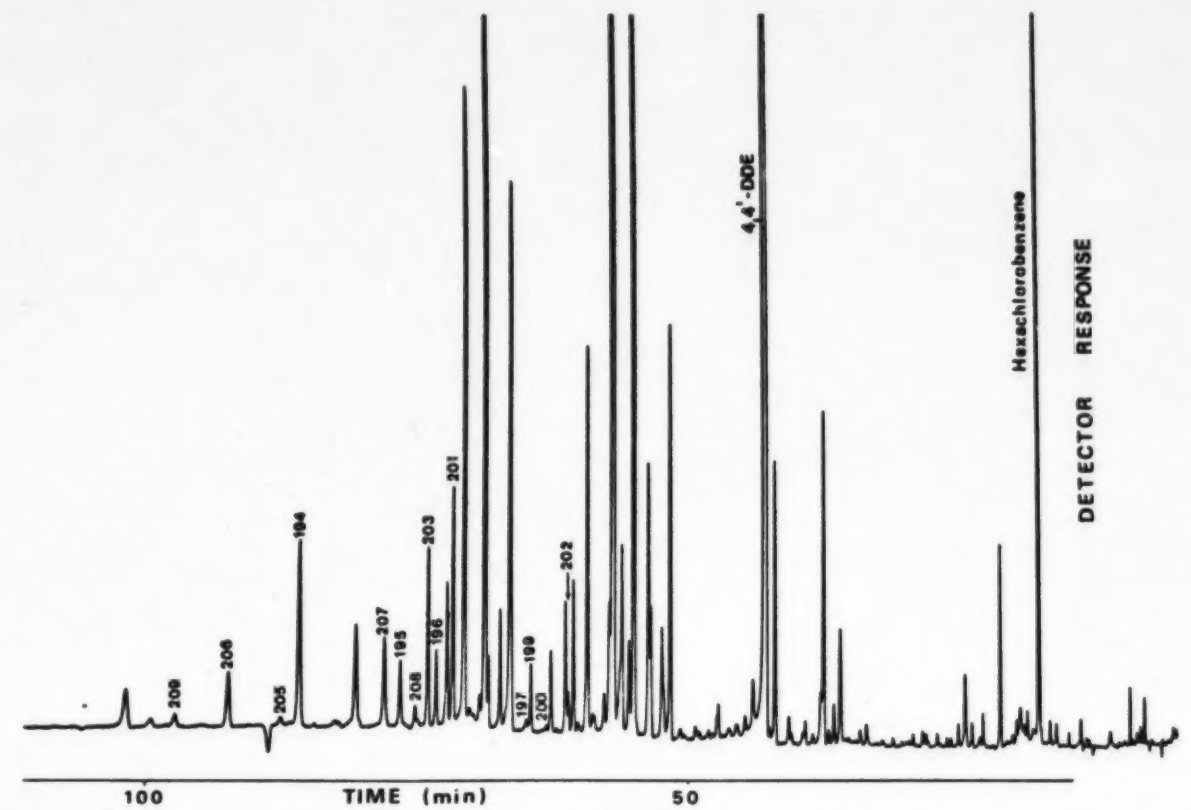

Figure 2. Glass capillary gas chromatogram of a human milk sample fraction (see text for instrumental operating conditions).

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